recovering stem cells from the feeder layer; and

growing the recovered stem cells under culture conditions that induce somatic differentiation, wherein the somatic cells generated are capable of transplantation to a patient in need, wherein a genetic modification to the congenital disease has been introduced into the cells capable of transplantation, and wherein said culture conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

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REMARKS

In the Office Action dated October 25, 2001, claims 1-36 are pending. Claims 1-18 and 29-36 are withdrawn from consideration as drawn to a non-elected invention. Claims 19-28 are currently under examination.

This Response addresses each of the Examiner's rejections and objections set forth in the Office Action. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

In the pending Office Action, Applicants' claim for foreign priority based on two Australian applications filed on November 9, 1998 and September 15, 1999, is acknowledged. However, the Examiner points out that certified copies of these applications have not been provided as required by 35 U.S.C. §119(b).

Applicants have provided herewith certified copies of these Australian applications. As such, Applicants respectfully submit that the requirements for claiming foreign priority under 35 U.S.C. §119(b) are met.

The specification is objected to in the Office Action. Specifically, the Examiner points out that the abstract is not presented as a single paragraph. In addition, the Examiner indicates that the specification has not been amended to insert the sequence identifiers.

Applicants have provided a new abstract in a single paragraph. In addition, the specification has been amended to insert the sequence identifiers. As such, withdrawal of the objection to the specification is respectfully requested.

Regarding the claims, Applicants have rewritten claim 19 to be an independent claim, drawn to an *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells. The amendment to claim 19 is supported by the entire specification and by original claims 8-17. Claims 20-26 depend upon claim 19. Claims 27-28 have been canceled without prejudice. Applicants reserve the right to pursue the subject matter of these canceled claims in a continuing application. Claims 37-46 have been added. Claims 37-44 are drawn to an *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells. Support for claims 37-44 is found throughout the specification and in original claims 8-28. Claims 45-46 are directed to a method of preventing and treating a congenital disease with somatic cells prepared from undifferentiated, pluripotent human embryonic stem cells. Support for claims 45-46 is found in the specification, e.g., at page 25, lines 8-23. No new matter is introduced.

In the pending Office Action, claims 19-28 are objected to because of the following informalities: claims 19-28 are dependent on non-elected claims; and the term 'according' in claim 22 is misspelled.

It is respectfully submitted that claims 19-28 as presently amended do not depend upon non-elected claims. Furthermore, the typographical error in claim 22 has been corrected. As such, the objection to claims 19-28 is overcome. Withdrawal of the objection is therefore respectfully requested.

Claims 19-28 are rejected under 35 U.S.C. §112, first paragraph, allegedly because the specification, while being enabling for a method of culturing a human pluripotent embryonic stem cell and deriving spontaneously differentiated cell types therefrom, does not reasonably provide ennoblement for culturing human totipotent embryonic stem cells or methods for the controlled differentiation of a stem cell.

In an effort to favorably advance the prosecution of the present application, Applicants have amended the claims to specifically refer to pluripotent stem cells. Applicants reserve the right to pursue the subject matter relating to totipotent embryonic stem cells in a continuing application.

As to differentiation of a stem cell into a somatic cell lineage, the Examiner acknowledges that the specification teaches methods in which stem cells are induced to differentiate. However, the Examiner argues that the specification teaches that, after the differentiation, selective cultivation or other means of isolation of differentiated cells from the resulting mixed population is practiced (page 23, lines 10-28 of the specification). The Examiner contends that the specification is silent with respect to methods in which only specific types of differentiated cells are generated. Relying upon Thomson *et al.* (Trends in Biotech, 18:53-57, 2000), the Examiner argues that the art recognizes that methods for differentiating embryonic stem cells into specific cell types are still not attainable.

Applicants respectfully submit that the present claims are expressly directed to methods of inducing the differentiation of pluripotent stem cells. In this regard, the present specification clearly teaches the culturing conditions that induce differentiation of undifferentiated stem cells. As shown in the specification at page 35-36, undifferentiated stem cells have been induced to differentiate, resulting in a mixture of cell types including neurofilaments, muscle, and embryoid bodies similar to those formed in mouse ES cell aggregates.

Applicants further submit that the fact that a controlled differentiation is not specifically exemplified in the specification does not warrant the conclusion that the claimed methods are not enabled. The claimed methods can be achieved with or without controlling the outcome of differentiation to a specific somatic lineage. In addition, Applicants are not required to exemplify each and every aspect of the claimed invention in order to satisfy the enablement requirement under 35 U.S.C. §112, first paragraph. Applicants need not include a specific working example in order to meet the enablement requirement. In re Anderson,176

USPQ 331, 333 (CCPA 1973). In fact, a reasonable belief that Applicants' success with one embodiment of the invention could be extrapolated to other embodiments by a skilled artisan is sufficient to support enablement. See, In re Wright, 999 F.2d.1557, 1564, 27 USPQ2d 1510, 1515 (Fed. Cir. 1993).

In view of the foregoing, it is respectfully submitted that the claimed methods are fully supported by an enabling disclosure. Withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is therefore respectfully requested.

Claims 19-28 are rejected under 35 U.S.C. 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner alleges that claim 19 is confusing for reciting conditions which "induce somatic differentiation" but which "do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages".

It appears that the Examiner questions (1) how there can be differentiation but not extraembryonic differentiation, and (2) whether the use of the term "unidirectional" in the claim is intended to mean that the claim encompasses extraembryonic lineages that are potentially capable of de-differentiation back into a stem cell.

Applicants respectfully submit that the difference between extraembryonic and somatic differentiation is understood by those of skilled in the art. Extraembryonic tissues, such as yolk sac and placenta, are tissues derived from the zygote and which play a supporting role during embryonic and fetal development, and are discarded at or before birth. Extraembryonic tissues do not contribute to the tissues of the new organism.

Applicants further submit that the development of extraembryonic tissues *in vitro* from ES cells may occur at the expense of stem cell renewal, or formation of desired types of body cells. Moreover, extraembryonic tissues in the embryo have multiple roles in signaling to the pluripotent cells to direct their fate; elaboration of these signals *in vitro* in a chaotic fashion may lead to undesirable outcomes such as stem cell extinction or formation of

unwanted cell types. Unidirectional differentiation into cells with the properties of extraembryonic endoderm (yolk sac and its precursors) occurs under some conditions of ES cell culture and will jeopardize formation of tissue cells.

In view of the foregoing, it is respectfully submitted that claim 19 is not contradictory in requiring that there be an induction of somatic differentiation, but no extraembryonic differentiation of the stem cells. In addition, those skilled in the art would understand that the phrase "unidirection" is not intended to encompass embodiments in which cells somehow de-differentiate from the extraembryonic lineages.

Regarding claim 20, the Examiner considers the phrase "a differentiated somatic lineage or multiple somatic lineage" to be indefinite, allegedly because it is unclear as to whether the same culturing condition is responsible for both a single lineage and multiple lineages.

Claim 20 has been amended to recite "a differentiated somatic lineage or multiple differentiated somatic lineages". It is respectfully submitted that claim 20 as presently amended is not indefinite.

As to claims 20-25, the Examiner considers that the phrase "a differentiation inducing fibroblast layer" renders the claims indefinite. The Examiner states that it is unclear whether the feeder cells themselves or the culturing conditions of the feeder layers result in the induction of differentiation.

It is respectfully submitted that "a differentiation inducing fibroblast layer" is a product of both the specific type of feeder layer and culture conditions used, as clearly defined by the specification. For instance, at page 20, lines 19-22, the specification provides that "without being restricted by theory, it has now become evident that the type and handling of the fibroblast feeder layer is important for maintaining the cells in an undifferentiated state or inducing differentiation of the stem cells". In addition, at page 20, lines 23 to 29, it is clear that it is a combination of correctly identifying the fibroblast feeding layers and culturing the cells at high density culture conditions which is integral to achieving the differentiation, as

presently claimed. Therefore, Applicants submit that the meaning of term "a differentiation inducing fibroblast layer" is clear in light of the specification.

The Examiner also considers claim 23 to be unclear and confusing, allegedly because the method of claim 19 is drawn to a method of somatic differentiation, however, claim 23 recites a test for fibroblast feeder cells which limit extraembryonic differentiation.

In view of the explanation hereinabove relating to the distinction of somatic differentiation and extraembryonic differentiation of the stem cells, it is respectfully submitted that claim 23 is clear and not indefinite.

With respect to claim 26, the Examiner contends that the recitation of isolation of "progenitor cells" is unclear, allegedly because the method is directed to preparing a culture of "differentiated somatic cells".

It is respectfully submitted that the claimed method is drawn to a method of inducing somatic differentiation. Progenitor cells are differentiated cells, but cells which are capable of further differentiation into a limited number of mature somatic cell lineages. Applicants respectfully direct the Examiner's attention to the sections of the specification where it is taught how to identify and isolate committed progenitor cells (e.g. page 23, lines 10 to 28).

Claims 27 and 28 are also rejected as allegedly vague and unclear.

It is respectfully submitted that claims 27-28 have been canceled without prejudice. Applicants reserve the right to pursue the subject matter of claims 27-28 in a continuing application.

Claims 19-28 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Thomson *et al.* (Science 282:1145-1147).

Applicants submit that the cancellation of claims 27-28 renders the rejection of these claims moot. Insofar as claims 19-26 are concerned, the Examiner contends that Thomson *et al.* (Science) teach human pluripotent embryonic cell lines and various culturing methods used to differentiate the cell lines. Among the parameters taught to affect

differentiation of the cell lines was the feeder layer, the cell density, and various growth factors. The Examiner indicates that Thomson et al. (Science) appear to have shown differentiation of the cells into various cell types, including neuronal cells (neural epithelium shown in Figure 4B). Thus, the Examiner concludes that Thomson *et al.* (Science), by describing methods to stimulate or allowing the cell lines to differentiate in culture, anticipate the methods of claims 19-26.

Applicants respectfully submit that the present claims are directed to in vitro methods for inducing differentiation. Thomson et al. (Science) do not disclose the generation of differentiated somatic lineages of non-extraembryonic type *in vitro*. The evidence cited by the Examiner shows the differentiation of the cell lines that were cultured *in vivo*, not *in vitro*. In particular, the cell types in a teratoma in Figure 4b of the publication, referenced by the Examiner, are not from *in vitro* culture. Therefore, it is respectfully submitted that Thomson et al. do not teach the claimed invention. Withdrawal of the rejection of the claims under 35 U.S.C. §102(b) based on Thomson *et al.* (Science) 282:1145-1147) is therefore respectfully requested.

Claims 19-28 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Thomson (US Patent No. 6,200,806).

Applicants respectfully submit that the differentiation of ES cells disclosed by the '806 patent appears to be extraembryonic in nature. See col.15, lines 10-26 and lines 50-57 of the '806 patent. Nowhere does the '806 patent teach methods which do not result in differentiation into extraembryonic lineages. Accordingly, the '806 patent does not anticipate the present claims. Withdrawal of the rejection of the claims under 35 U.S.C. §102(e) based on the '806 patent is therefore respectfully requested.

Claims 27 and 28 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Kaufman *et al.* (US Patent 6,280,718). Claims 27-28 are also rejected under 35 U.S.C. §102(b) as allegedly anticipated by Damjanov *et al.* (Lab. Invest. 68:220-232).

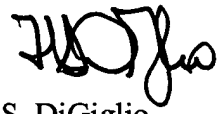
In view of the cancellation of claims 27-28, the rejections of these claims are rendered moot. Withdrawal thereof is therefore respectfully requested.

It is also observed that the filing date of the '718 patent is 8 November 1999, which is after the priority applications of the present application were filed (on November 9, 1998 and September 15, 1999, respectively). Given that certified copies of these applications are provided herewith, the '718 patent is not a proper prior art reference under 35 U.S.C. §102(e).

Attached hereto is a marked-up version of the changes made to the specification and claims by the instant amendment. The attached page is captioned "**Version with Markings to Show Changes Made.**"

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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FSD/XZ:ab
Encls.:

- Certified copies of priority documents
- Version with Markings to Show Changes Made

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 31, line 18 as follows:

--To monitor expression of Oct-4, RT-PCR was carried out on colonies consisting predominantly of stem cells, or colonies which had undergone spontaneous differentiation as described below. mRNA was isolated on magnetic beads (Dynal AS, Oslo) following cell lysis according to the manufacturer's instructions, and solid-phase first strand cDNA synthesis was performed using Superscript II reverse transcriptase (Life Technologies). OCT-4 transcripts were assayed using the following primers: 5'-CGTTCTCTTTGGAAAGGTGTTC (forward) (SEQ ID NO: 1) and 3'-ACACTCGGACCACGTCTTTC (reverse) (SEQ ID NO: 2). As a control for mRNA quality, betaactin transcripts were assayed using the same RT-PCR and the following primers: 5'-CGCACCACTGGCATTGTCAT-3' (forward) (SEQ ID NO: 3), 5'-TTCTCCTTGATGTCACGCAC-3' (reverse) (SEQ ID NO: 4). Products were analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining.--

Please amend the paragraph beginning at page 32, line 19 as follows:

--Clusters of cells destined to give rise to neural precursors were identified by their characteristic morphological features in central areas of ES cell colonies 2-3 weeks after plating. The clusters were dissected mechanically by a micropipette and replated in fresh serum free medium. Within 24 hours they formed spherical structures. The expression of the transcription factor PAX-6 and the intermediate filament nestin by these clusters was

demonstrated by RT-PCR as described above. The following primers were used for PAX-6 and nestin respectively: Pax-6 forward primer, 5'AACAGACACAGCCCTCACAAACA3' (SEQ ID NO: 5); Pax-6 reverse primer, 5'CGGGAACTTGAACTGGAACTGAC3'(SEQ ID NO: 6); nestin forward primer, 5'CAGCTGGCGCACCTCAAGATG3' (SEQ ID NO: 7); nestin reverse primer, 5'AGGGAAGTTGGGCTCAGGACTGG3' (SEQ ID NO: 8).--

Please amend the abstract as follows:

--The present invention relates to undifferentiated human embryonic stem cells, methods of cultivation and propagation, production of differentiated cells and in particular the production of human [ES] embryonic stem cells capable of yielding somatic differentiated cells *in vitro*, as well as committed progenitor cells capable of giving rise to mature somatic cells and uses thereof. [----------In one aspect of the] The present invention[, there is provided] also provides a purified preparation of undifferentiated human embryonic stem cells capable of proliferation *in vitro*. [---------- -In another aspect, there is provided] Furthermore, the present invention provides a somatic cell differentiated *in vitro* from an undifferentiated embryonic stem cell. There is also provided a committed progenitor cell capable of giving rise to mature somatic cells.--

IN THE CLAIMS:

Please cancel claims 27 and 28 without prejudice.

Please amend the claims as follows:

19. (Amended) [A method according to any one of claims 8 to 17] An *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells, wherein said undifferentiated, pluripotent human embryonic stem cells are prepared by a process comprising:

obtaining an *in vitro* fertilised human embryo and growing said embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from said embryo;

culturing said ICM cells under conditions which do not induce extraembryonic differentiation and cell death and promote proliferation of undifferentiated stem cells; and

recovering stem cells;

[further including the step of] said method comprising growing said stem cells under culture conditions that induce somatic differentiation, [and] wherein said conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

20. (Amended) A method according to claim 19 wherein [the condition includes] said culture conditions comprise prolonged cultivation of the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer to induce a differentiated somatic lineage or multiple differentiated somatic [lineage] lineages.

22. (Amended) A method [accordin] according to claim 20 or 21 wherein [the] said fibroblast feeder layer comprises embryonic fibroblasts.

23. (Amended) A method according to [any one of claims] claim 20 [to 22] or 21 wherein the fibroblasts are tested for their ability to promote embryonic stem cell growth and to limit extraembryonic differentiation.

24. (Amended) A method according to any one of claims 19[to 23], 20 or 21 wherein the [embryonic] fibroblasts are prepared and tested for their ability to allow somatic differentiation of embryonic stem cells.

25. (Amended) A method according to any one of claims 19[to 24], 20 or 21 wherein [the culture condition includes] said culture conditions comprise cultivating the cells for prolonged periods and/or at high density in the presence of a differentiation inducing fibroblast feeder layer to induce somatic differentiation.

26. (Amended) A method for the isolation of committed progenitor cells from a culture of differentiated cells, said method comprising:

preparing a culture of differentiated cells according to any one of claims 19 [to 25], 20 or 21; and

isolating committed progenitor cells from the culture.

Please add the following claims:

37. An *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells, wherein said undifferentiated, pluripotent human embryonic stem cells are prepared by a process comprising:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cell mass (ICM) cells from the embryo;

culturing ICM cells on a fibroblast feeder layer to obtain proliferation of undifferentiated stem cells; and

recovering the stem cells from the feeder layer;

said method comprising growing the stem cells under culture conditions that induce somatic differentiation, wherein said conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

38. A method according to claim 37 wherein said culture conditions comprise prolonged cultivation of the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer to induce a differentiated somatic lineage or multiple differentiated somatic lineages.

39. A method according to claim 38 wherein said differentiation inducing fibroblast feeder layer is at least one of a mouse fibroblast feeder layer or human fibroblast feeder layer.

40. A method according to claim 38 or 39 wherein said fibroblast feeder layer comprises embryonic fibroblasts.

41. A method according to claim 38 or 39 wherein the fibroblasts are tested for their ability to promote embryonic stem cell growth and to limit extraembryonic differentiation.

42. A method according to any one of claims 37, 38 or 39 wherein the embryonic fibroblasts are prepared and tested for their ability to allow somatic differentiation of embryonic stem cells.

43. A method according to any one of claims 37, 38 or 39 wherein said culture conditions comprise cultivating the cells for prolonged periods and/or at high density in the presence of a differentiation inducing fibroblast feeder layer to induce somatic differentiation.

44. A method for the isolation of committed progenitor cells from a culture of differentiated cells, said method comprising:

preparing a culture of differentiated cells according to any one of claims 37, 38 or 39; and

isolating committed progenitor cells from the culture.

45. A method of preventing and treating a congenital disease, said method including:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death and promote proliferation of undifferentiated stem cells;

recovering stem cells; and

growing the recovered stem cells under culture conditions that induce somatic differentiation, wherein the somatic cells generated are capable of transplantation to a patient in need, wherein a genetic modification to the congenital disease has been introduced into the cells capable of transplantation, and wherein said culture conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

46. A method of preventing and treating a congenital disease, said method comprising:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cell mass (ICM) cells from the embryo;

culturing ICM cells on a fibroblast feeder layer to obtain proliferation of undifferentiated stem cells;

recovering stem cells from the feeder layer; and

growing the recovered stem cells under culture conditions that induce somatic differentiation, wherein the somatic cells generated are capable of transplantation to a patient in need, wherein a genetic modification to the congenital disease has been introduced into the cells capable of transplantation, and wherein said culture conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.